

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 July 2001 (26.07.2001)

PCT

(10) International Publication Number
WO 01/52883 A1

- (51) International Patent Classification⁷: **A61K 38/55**
- (21) International Application Number: PCT/US01/01968
- (22) International Filing Date: 19 January 2001 (19.01.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/177,137 20 January 2000 (20.01.2000) US
60/203,357 10 May 2000 (10.05.2000) US
09/728,404 28 November 2000 (28.11.2000) US
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- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 01/52883 A1

(54) Title: INHIBITORS OF PROTEASE-ACTIVATED RECEPTOR-2 (PAR-2) AS NOVEL ASTHMA THERAPEUTICS

(57) Abstract: The present invention relates to a method of treating bronchial hyperresponsiveness by inhibiting the activation of proteinase-activated receptor-2. The present invention also relates to a method of evaluating molecules for their ability to inhibit the activation of proteinase-activated receptor-2.

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Protease-activated receptor-2 (PAR-2) is a G protein-coupled receptor possessing seven transmembrane domains (Nystedt et al., *Proc. Natl. Acad. Sci. USA*, 1994; 91:9208-9212) present on airway smooth muscle cells. PAR-2 is activated by the proteolytic action of trypsin and tryptase. Proteolytic cleavage of the extracellular N-terminal domain of PAR-2 (e.g., by mast cell tryptase Molino et al., *J. Biol. Chem.* 1997; 272:4043-4049) exposes a tethered sequence which binds to and activates the transmembrane region of the receptor. Further descriptions of PAR-2 are included in the following documents: Hadasit Medical Research Services & Development, Antisense or antibody treatment of metastatic tumors (WO 200008150) and Antisense treatment of hypoxia and ischemia (WO 9942483); Johnson & Johnson, Antagonist or protease inhibitor for skin pigmentation (WO 9904752) and Antagonist or protease inhibitor to block ICAM-1 expression (WO 9930729); and University of California, Antagonist or protease inhibitor for post operative ileus (WO 9843477).

H₂N-Ser-Leu-Ileu-Gly-Arg-Leu-CONH₂ (SLIGRL), the peptide corresponding to the tethered ligand exposed following tryptase-mediated cleavage, has been shown to be a potent agonist of the PAR-2 receptor in several cellular systems. Cellular responses to tryptase and PAR-2 activating peptides include induction of
5 phosphatidylinositol hydrolysis and calcium mobilization.

SUMMARY OF THE INVENTION

10 The present invention identifies materials and methods for treating bronchial hyperresponsiveness by inhibiting the activation of proteinase-activated receptor-2 (PAR-2). Such materials and methods may be used in the treatment of asthma, allergy, bronchitis and other conditions involving airway hyperresponsiveness. In addition, such materials and methods may be used in the treatment of other chronic or acute
15 airway inflammatory disorders associated with the activation of PAR-2 receptors.

Methods of inhibiting PAR-2 activation may include, but are not limited to, the inhibition of tryptase, the blockade of the proteolytic site on the extracellular receptor domain, the prevention of the interaction of the tethered peptide ligand with the receptor, and the inhibition of signal transduction pathways induced by receptor-ligand
20 interactions. Therapeutic molecules may include protein (such as secretory leukocyte protease inhibitor), peptide, small molecule, and antibody moieties. The inhibition of tryptase by secretory leukocyte protease inhibitor (SLPI) and small molecule tryptase inhibitors and/or the antagonism of PAR-2 autoactivation following tryptase cleavage by small molecules, peptides or antibodies may be effective methods of therapeutic
25 intervention in asthma.

In another aspect, the present invention involves a method for identifying molecules useful in the treatment of airway hyperresponsiveness. The method involves contacting a molecule to a cell expressing a PAR-2, wherein the molecule is being evaluated as a potential PAR-2 antagonist or inhibitor, and determining whether PAR-2
30 autoactivation is inhibited or antagonized. A method for identifying a PAR-2 antagonist or inhibitor involves contacting a molecule to a cell expressing PAR-2 and

determining whether proteolytic cleavage and activation of PAR-2 is inhibited or antagonized. Possible mechanisms of action include, but are not limited to, blocking a protease which activates PAR-2, blocking the cleavage site on PAR-2, and blocking the self-binding of PAR-2.

- 5 Uses for such therapies will include the treatment of asthma and other chronic or acute airway inflammatory disorders which are associated with the activation of PAR-2 receptors. Disorders involving cell types expressing PAR-2 (including, but not limited to, endothelial cells, keratinocytes, aorta, coronary artery, intestinal arteries, smooth muscle and epithelium, sweat glands, vascular smooth muscle, astrocytes, T-
10 lymphocytes) may be treated by PAR-2 inhibition.

BRIEF DESCRIPTION OF THE FIGURES

- 15 Figure 1 depicts the activation of the PAR-2 receptor promotes histamine hyperresponsiveness in bronchial cells.

Figure 2 depicts the dose-dependent stimulation of histamine hyperresponsiveness by the PAR-2 agonist SLIGRL.

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Figure 3 depicts the time-dependent stimulation of histamine hyperresponsiveness by the PAR-2 agonist SLIGRL.

- Figure 4 depicts the effect of SLPI and APC-366 on SLIGRL-stimulated airway
25 hyperresponsiveness.

Figure 5 depicts the effect of indomethacin on the induction of bronchi hyperresponsiveness.

- 30 Figure 6 depicts the effect of PAR-2 agonists on the stimulation of interleukin-8 (IL-8) production in endothelial cells.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

5 The present study investigates tryptase-mediated activation of proteinase-activated receptor-2 (PAR-2) in promoting airway hyperresponsiveness. Synthetic peptides corresponding to the structure of the tethered ligands for the PAR receptors were assessed for the ability to promote the development of hyperresponsiveness in bronchial cells. It has been found that a soluble peptide derived from the protease-
10 exposed tethered sequence (e.g., Ser-Leu-Ile-Gly-Arg-Leu-NH₂ (SLIGRL) promotes PAR-2 activation.

 We propose that activation of PAR-2 by tryptase released by lung mast cells contributes to the pathology of asthma. We have previously demonstrated that treatment of guinea pig bronchi with tryptase results in the development of
15 hyperresponsiveness, a primary pathophysiologic response associated with asthma. Development of tryptase-induced hyperresponsiveness is blocked by small molecule tryptase inhibitors and secretory leukocyte protease inhibitor (SLPI) (*Life Sci.* 1998; 63:2295-2303). In addition, it is now shown that the treatment of bronchial rings with SLIGRL promotes hyperresponsiveness to the contractile mediator histamine. In
20 contrast, treatment with the inactive peptide Leu-Ser-Ile-Gly-Arg-Leu-NH₂ (LSIGRL) fails to promote airway hyperresponsiveness. These results suggest that inhibitors of PAR-2 activation may have utility as asthma therapeutics.

25

Example 1

MATERIALS AND METHODS

Tissue preparation: Male Hartley guinea pigs (500-800 g) were sacrificed by asphyxiation using CO₂. The lungs were removed and placed in cold oxygenated (95%
30 O₂ + 5% CO₂) Krebs solution (pH 7.4). The bronchi were dissected and cleaned of

excess fat and connective tissue. Bronchial segments, measuring 2 mm in length, were suspended in a 25 ml tissue bath at 37° C, filled with Krebs oxygenated solution. Tissues were equilibrated for one hour and then washed once. Tissues were stretched to a resting tension of 300 mg for one hour in the presence of the adrenergic antagonist
5 phentolamine (10^{-5} M), the cyclooxygenase inhibitor indomethacin (10^{-6} M) and the beta adrenergic receptor agonist isoproterenol (10^{-7} M). After a stable baseline was achieved, tissues were incubated with PAR receptor agonists. The effects of the agonists on smooth muscle responsiveness were evaluated by assessing the tissue's subsequent response to histamine (10^{-8} to 3×10^{-4} M). Tissues were then washed at least
10 twice during a period of one hour until they returned to baseline and then recontracted maximally to histamine (3×10^{-4} M).

Experimental procedure: Tissues were exposed to different peptide concentrations (0.1 to 20 μ M) for varying exposure times (15 to 50 min) to study concentration and
15 incubation time effects. Each isolated segment received a single concentration of the peptide for a period of time followed by histamine concentration response curve as described above.

The isometric contractions of the bronchial segments were recorded using a Radnotti isometric force transducer connected to a MacLab analog to digital converter
20 system and a Macintosh Performa 630 CD computer. Statistical significance of dose response data was determined by ANOVA followed by Fisher's PLSD and Bonferroni/Dunn analysis.

Experimental results: *Ex vivo* exposure of guinea pig bronchi to the PAR-2 agonist
25 $\text{H}_2\text{N-Ser-Leu-Ile-Gly-Arg-Leu-CONH}_2$ (SLIGRL) (10^{-7} to 10^{-5} M) induced a concentration-dependent increase of contractile response to histamine. Treatment with 10^{-5} M SLIGRL for 45 minutes increased subsequent responsiveness to histamine (3×10^{-4} M) by 54 ± 3 % ($p < 0.05$ versus buffer-treated control). Effects of the peptide were observed following at least a 30 minutes preincubation with the tissue. The inactive
30 analogue $\text{H}_2\text{N-Leu-Ser-Ile-Gly-Arg-Leu-CONH}_2$ (10^{-5} M; LSLIGRL) failed to promote hyperresponsiveness. In contrast, the PAR-1 agonist peptide $\text{H}_2\text{N-Ser-Phe-Leu-Leu-}$

Arg-Asn-CONH₂ (SFLLRN) did not promote significant airway hyperresponsiveness. These results demonstrate the potential involvement of tryptase-mediated activation of PAR-2 in promoting airway hyperresponsiveness.

5 Figure 1 illustrates that the activation of the PAR-2 receptor promotes histamine hyperresponsiveness in isolated guinea pig bronchi. Isolated bronchi treated with 10 μ M SLIGRL (PAR-2 agonist), LSIGRL (inactive PAR-2 analog), or SFLLRN (PAR-1 agonist) were subsequently contracted with histamine (0.3 mM). Bronchial response to histamine is expressed as maximum contractile response (g, mean \pm s.e.m.)(*p<0.05 vs. histamine response of control tissue, n = 4-11).

10 Figure 2 illustrates the dose-dependent stimulation of histamine hyperresponsiveness by the PAR-2 agonist SLIGRL. Panel A: Isolated bronchi treated with SLIGRL were subsequently contracted with histamine. Bronchial response to histamine is expressed as maximum contractile response (g, mean \pm s.e.m.)(*p<0.05 vs. histamine response of control tissue, n = 3-6). Panel B depicts the bronchial response to histamine expressed as a percentage of maximum histamine contractile response (g, mean \pm s.e.m.) (n = 3-6).

20 Figure 3 depicts the time-dependent stimulation of histamine hyperresponsiveness by the PAR-2 agonist SLIGRL. Isolated bronchi treated with 10 μ M SLIGRL were subsequently contracted with histamine (0.3 mM). Bronchial response to histamine is expressed as maximum contractile response (g, mean \pm s.e.m.)(*p<0.05 vs. histamine response of control tissue, n = 3-6).

25 Figure 4 illustrates the effect of SLPI and APC-366 (tryptase inhibitors) on SLIGRL-stimulated airway hyperresponsiveness. Isolated bronchi treated with 10 μ M SLIGRL alone or in the presence of SLPI (3 μ M) or APC-366 (3 μ M) were subsequently contracted with histamine (0.3 mM). Bronchial response to histamine is expressed as maximum contractile response (g, mean \pm s.e.m.)(*p<0.05 vs. histamine response of control tissue, n = 3-11). These results contrast with the ability of these protease inhibitors to block tryptase-induced hyperresponsiveness. These results

indicate that protease inhibition has no therapeutic effect subsequent to the proteolytic activation of PAR-2.

Figure 5 illustrates the effect of indomethacin on the induction of guinea pig bronchi hyperresponsiveness. Isolated bronchi treated with 10 μ M SLIGRL or
5 SFLLRN in the presence or absence of indomethacin (10^{-6} M) were subsequently contracted with histamine (0.3 mM). Bronchial response to histamine is expressed as maximum contractile response (g, mean \pm s.e.m.)(* $p < 0.05$ vs. histamine response of control tissue, n = 2-11).

10 The synthetic peptide SLIGRL induced an increased responsiveness of bronchial smooth muscle contractility to histamine in a manner similar to that observed when this tissue was exposed to the mast cell serine protease tryptase. The inactive analogue LSIGRL and the PAR-1 agonist SFLLRN had no effect on bronchial contractility in response to histamine.

15 The effect of indomethacin on the PAR-2-mediated response may reflect a prostaglandin-mediated relaxation mechanism mediated by airway epithelium. The disruption of the epithelial layer as a consequence of airway remodeling in asthma may result in increased susceptibility to PAR-2 activation.

The results of this study support a model of asthma in which release of tryptase
20 from activated mast cells impacts the pathophysiology of the disease through proteolytic cleavage and activation of PAR-2 receptors in the airway. Thus, the activation of PAR-2 receptor may be a significant mechanism in the development of airway hyperresponsiveness in asthma, and the inhibition of this PAR-2-mediated pathway may provide a potential new therapeutic target for the treatment of asthma.
25 The inhibition of tryptase by SLPI or small molecule tryptase inhibitors and antagonism of PAR-2 autoactivation following tryptase cleavage may be effective methods of therapeutic intervention in asthma.

Example 2

PAR-2 MEDIATES ACTIVATION OF ENDOTHELIAL CELLS

The mast cell serine protease tryptase has been implicated as a critical mediator of airway inflammation associated with asthma. Tryptase may function, in part, through the activation of PAR-2. In this study, we examined the role of PAR-2 in promoting pro-inflammatory responses of endothelial cells.

Cell culture. Fourth passage human umbilical vein endothelial cells (HUVEC) (Clonetics, San Diego, CA) were grown to confluence in 96-well tissue culture-treated microtiter plates using endothelial growth media (EGM) (Clonetics) with 5% fetal bovine serum (Hyclone, Logan, UT) at 37°C with 5% CO₂. On the day before assay, the media was removed and replaced with 200 µL of fresh EGM with 5% fetal bovine serum.

Interleukin-8 production. On the day of the assay, 25 µM of a test compound (for example, antibody, peptidomimetic, protein, peptide or small molecule) was added to appropriate wells of the microtiter plates containing HUVECs. EGM was added to wells reserved for unstimulated and stimulated controls. After a 15 minute preincubation at 37°C, 25 µL of a 100 µM solution of the PAR-2 agonist SLIGRL was added to all wells except those reserved for unstimulated controls. Final volume in each well was 250 µL. After a four hour incubation at 37°C, the reaction plates were centrifuged at 150xG for five minutes, and 150 µL of supernatant was harvested from each well for IL-8 assessment. IL-8 was quantified using an ELISA kit obtained from Biosource International (Camarillo, CA).

Results. The PAR-2 agonists SLIGRL and tc-LIGRLO stimulated IL-8 production and ICAM-1 expression by HUVECs in a time and dose-dependent manner. Four hour treatment of HUVECs with SLIGRL or tc-LIGRLO (10 µM) induced 2.1 and 2.4 fold increase in IL-8 production and 1.3 and 1.4 fold increase in ICAM-1 expression

($p < 0.05$ vs. unstimulated control). In contrast, the inactive analogue H_2N -Leu-Ser-Ile-Gly-Arg-Leu- $CONH_2$ (LSIGRL) had no stimulatory effect. In comparison, tumor necrosis factor- α (TNF α , 0.59 nM) induced a 2.6 fold increase in IL-8 production and a 3.2 fold increase in ICAM-1 expression. TNF α (8 hours) stimulated a 3.5 fold increase in VCAM-1 expression while PAR-2 agonists had no effect. The PAR-1 (thrombin receptor) agonist H_2N -Ser-Phe-Leu-Leu-Arg-Asn- $CONH_2$ (SFLLRN) also stimulated the IL-8 and ICAM-1 response by 4.2 and 1.4% ($p < 0.05$). Co-treatment with PAR-2 agonist and TNF α resulted in the synergistic stimulation of IL-8 production.

The results of this study are further illustrated in Figure 6. PAR-2 agonists stimulate IL-8 production by human umbilical vein endothelial cells. Endothelial cells were treated as described above, and cell activation was assessed by the production of IL-8 (expressed as fold increase for stimulated cells versus unstimulated cells)(mean \pm standard error, $n=3-7$). In comparison, TNF α (0.1 μ g/ml) stimulated 3.36 ± 0.47 fold increase in IL-8 production. These results demonstrate the potential role of PAR-2 activation in inflammatory diseases and an useful assay for the evaluation of molecules which may inhibit PAR-2 activation.

Based upon these results it is appreciated that methods of inhibiting PAR-2 activation may include, but are not limited to, the inhibition of tryptase, the blockade of the proteolytic site on the extracellular receptor domain, the prevention of the interaction of the tethered peptide ligand with the receptor, and the inhibition of signal transduction pathways induced by receptor-ligand interactions. One mode of inhibition includes inhibitors of tryptase, such as small molecule inhibitors or protein-based inhibitors such as (but not limited to) SLPI. Another mode involves the use of antibodies, peptides, or small molecule antagonists to block the proteolytic activation site, tethered ligand, or receptor-ligand interaction, and thereby inhibit PAR-2 activation and/or function.

Uses for such therapies will include the treatment of asthma and other chronic or acute airway inflammatory disorders associated with mast cell activation. Disorders

involving cell types expressing PAR-2, including endothelial cells, keratinocytes, aorta, coronary artery, intestinal arteries, smooth muscle and epithelium, sweat glands, vascular smooth muscle, astrocytes, T-lymphocytes may also be treated by PAR-2 inhibition.

5 It will be appreciated by those skilled in the art that the antibody, protein, peptide, or small molecule may be administered parenterally, *e.g.*, by injection intravenously, intraperitoneally, intramuscularly, intrathecally or subcutaneously. Alternatively, the selected molecule may be formulated as a composition for oral administration (including sublingual and buccal), pulmonary administration (intranasal
10 and inhalation), topical administration, transdermal administration, and rectal administration. Delivery may involve a single dose schedule or a multiple dose schedule.

 The present invention also provides for stable gene expression in a host, or modulating gene expression in a host, which comprises administering a vector or
15 vectors which will result in the *in vivo* expression of the selected therapeutic molecule. The "effective amount" of the vector or therapeutic molecule is such as to produce the desired effect in a host which can be monitored using several end-points known to those skilled in the art. For example, effective nucleic acid transfer to a host cell could be monitored in terms of a therapeutic effect (*e.g.* alleviation of some symptom associated
20 with the disease or syndrome being treated), or by further evidence of the transferred gene or coding sequence or its expression within the host (*e.g.*, using the polymerase chain reaction, Northern or Southern hybridizations, or transcription assays to detect the nucleic acid in host cells, or using immunoblot analysis, antibody-mediated detection, or particularized assays to detect protein or polypeptide encoded by the transferred
25 nucleic acid, or impacted in level or function due to such transfer). One such particularized assay includes an assay for expression of a reporter or marker gene.

 The pharmaceutical compositions containing the vector or therapeutic molecule typically include a therapeutically or prophylactically effective amount of the vector or therapeutic molecule in admixture with one or more pharmaceutically and
30 physiologically acceptable formulation agents selected for suitability with the mode of administration. Suitable formulation materials or pharmaceutically acceptable agents

include, but are not limited to, antioxidants, preservatives, diluting agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, and diluents. For example, a suitable vehicle may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to a formulation agent(s) suitable for accomplishing or enhancing the delivery of the vector or therapeutic molecule as a pharmaceutical composition.

10 The primary solvent in a composition may be either aqueous or non-aqueous in nature. In addition, the vehicle may contain other formulation materials for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the composition may contain additional formulation materials for modifying or maintaining the rate of release of the vector or therapeutic molecule, or for promoting the absorption or penetration of the vector or therapeutic molecule.

When systemically administered, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such pharmaceutically acceptable solutions, with due regard to pH, isotonicity, stability and the like, is within the skill of the art.

Therapeutic formulations of the vector or therapeutic molecule compositions useful for practicing the present invention may be prepared for storage by mixing the selected composition having the desired degree of purity with optional physiologically acceptable stabilizers (*Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company, 1990). Acceptable stabilizers preferably are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and preferably include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose,

mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

The optimal pharmaceutical formulation will be determined by one skilled in
5 the art depending upon the intended route of administration, delivery format and desired dosage. See for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (Mack Publishing Co., Easton, PA 18042 pages 1435-1712, 1990.)

An effective amount of an the vector or therapeutic molecule composition to be employed therapeutically will depend, for example, upon the therapeutic objectives
10 such as the indication for which the vector or therapeutic molecule is being used, the route of administration, and the condition of the patient. Accordingly, it may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, a clinician will administer the composition until a transgene dosage is reached that achieves the desired effect.
15 The composition may therefore be administered as a single dose, or as two or more doses over time, or as a continuous infusion via implantation device or catheter.

As further studies are conducted, information will emerge regarding appropriate dosage levels for the treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, the type of disorder under
20 treatment, the age and general health of the recipient, will be able to ascertain proper dosing.

A particularly suitable vehicle for parenteral injection is sterile distilled water in which the vector or therapeutic molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation may involve the formulation of the vector
25 or therapeutic molecule with an agent, such as injectable microspheres, bio-erodible particles or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection.

The preparations of the present invention may include other components, for example parenterally acceptable preservatives, tonicity agents, cosolvents, wetting
30 agents, complexing agents, buffering agents, antimicrobials, antioxidants and surfactants, as are well known in the art. For example, suitable tonicity enhancing

agents include alkali metal halides (preferably sodium or potassium chloride), mannitol, sorbitol and the like. Suitable preservatives include, but are not limited to, benzalkonium chloride, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid and the like. Hydrogen peroxide may also be used as preservative. Suitable cosolvents are for example glycerin, propylene glycol and polyethylene glycol. Suitable complexing agents are for example caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin. Suitable surfactants or wetting agents include sorbitan esters, polysorbates such as polysorbate 80, tromethamine, lecithin, cholesterol, tyloxapal and the like. The buffers can be conventional buffers such as borate, citrate, phosphate, bicarbonate, or Tris-HCl.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

A pharmaceutical composition may be formulated for inhalation. For example, the vector or therapeutic molecule may be formulated as a dry powder for inhalation. Alternatively, inhalation solutions may be formulated in a liquefied propellant for aerosol delivery. In yet another formulation, solutions may be nebulized.

Regardless of the manner of administration, the specific dose may be calculated by the delivery of a gene that produces a therapeutic effect according to body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The term "gene transfer" or "gene delivery" is used to refer to methods and/or systems for reliably inserting a particular polynucleotide vector (*e.g.*, DNA) into a target cell. Gene transfer may take place *in vivo* (*e.g.*, adeno-associated virus gene therapy) or *ex vivo* (*e.g.*, as with extracellular modification of cells with a retrovirus followed by transfer or implantation of the transformed cells into the host). Such

methods may result in the integration of the transferred genetic material into the genome of target cells or the transferred genetic material may function independently of the host cell genome.

Cell therapy or *ex vivo* gene therapy, *e.g.*, implantation of cells containing the desired DNA constructs of the present invention, is also contemplated. This embodiment would involve implanting cells containing the DNA constructs by which the expression of the gene of interest is then regulated. In order to minimize a potential immunological reaction, it is preferred that the cells be of human origin and produce a human gene of interest. It is envisioned, however, that the vectors may be used to modify heterologous donor cells and xenogeneic cells, as well as autologous cells, for delivery or implantation.

In some cases, vectors may be delivered through implanting into patients certain cells that have been genetically engineered to express and secrete the desired protein, antibody or polypeptide. Such cells may be animal or human cells, and may be derived from the patient's own tissue (autologous) or from another source, either human (allogeneic) or non-human (xenogeneic). Optionally, the cells may be immortalized. In order to further decrease the chance of an immunological response, the cells may be encapsulated to avoid the infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow release of the protein product(s) but prevent destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be accomplished without undue experimentation. For example, Baetge *et al.* (WO 9505452; PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down regulation *in vivo* upon implantation into a mammalian host. The devices

provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Numbers 4,892,538, 5,011,472, and 5,106,627. A system for encapsulating living cells is described in PCT Application WO 9110425 of Aebischer *et al.* See also, PCT Application WO 9110470 of Aebischer *et al.*, Winn
5 *et al.*, *Exper. Neurol.* 113:322-329, 1991, Aebischer *et al.*, *Exper. Neurol.* 111:269-275, 1991; and Tresco *et al.*, *ASAIO* 38:17-23, 1992.

The DNA constructs for the desired molecules described herein can be incorporated into a variety of vectors for introduction into cells. Suitable vectors
10 include, but are not restricted to, naked DNA, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), viral RNA vectors (such as retroviral or alphavirus vectors) and non-viral vectors (such as DNA complexed with cationic lipids or packaged within liposomes). It will be appreciated by those skilled in the art that an expression vector will also include a) a transcription initiation region; b)
15 a transcription termination region, and c) expression control sequences. It will also be appreciated that the DNA constructs and vectors may be produced by joining separately produced components.

Vectors containing the DNA constructs of the present invention may be delivered to cells by a variety of plasmid and non-viral delivery methods known to
20 those familiar with the art, including, but not restricted to, liposome-mediated transfer or lipofection, by incorporation into other delivery vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, naked DNA delivery (direct injection or direct uptake), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate mediated transformation, microinjection,
25 osmotic shock, microparticle bombardment (*e.g.*, gene gun), bio-chip materials and combinations of the above. Delivery materials and methods may also involve the use of components including, but not limited to, inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to
30 identify transformed cells, negative selection systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, transcription

factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 4,970,154 electroporation techniques; WO 9640958 nuclear ligands; U.S. Patent No. 5,679,559 concerning a lipoprotein-containing system
5 for gene delivery; U.S. Patent No. 5,676,954 involving liposome carriers; U.S. Patent No. 5,593,875 concerning methods for calcium phosphate transfection; and U.S. Patent No. 4,945,050 wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells.

10 Suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346
15 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells which have been treated *in vitro* to insert a DNA segment encoding a therapeutic molecule. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 5,631,236 involving adenoviral vectors; U.S. Patent No. 5,672,510 involving retroviral vectors; and U.S.
20 Patent No. 5,635,399 involving retroviral vectors expressing cytokines.

Preferably, the vectors persist in the cells to which they are delivered. Alternatively, some vectors may be used that provide for transient expression of the DNA constructs. Such vectors might be repeatedly administered as necessary.

The control or regulation of gene expression is also a highly desired objective in
25 the fields of protein production, diagnostics, transgenics, cell therapy and gene therapy. A variety of expression control systems have been described as means to transcriptionally control the expression of a transgene in a recipient host cell. Control means or gene switches include, but are not limited to, the following systems.

Rapamycin and "rapalogs" may be used to dimerize chimeric proteins which
30 contain a small molecule-binding domain and a domain capable of initiating a

biological process, such as a DNA-binding protein or transcriptional activation protein (as described in WO 9641865 (PCT/US96/099486); WO 9731898 (PCT/US97/03137) and WO 9731899 (PCT/US95/03157)). The dimerization of the proteins can be used to initiate transcription of the transgene.

5 An alternative regulation technology uses a method of storing proteins, expressed from the gene of interest, inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain which results in the retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can
10 be released, however, by administering a drug (*e.g.*, small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. See *Science* 287:816-817 and 826-830, 2000.

Mifepristone (RU486) is used as a progesterone antagonist. The binding of a
15 modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors which then pass into the nucleus to bind DNA. The ligand binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. Patent No. 5,364,791; WO 9640911 and
20 WO 9710337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation
25 domain/DNA-binding domain/ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. Patent No. 5,514,578; WO 9738117; WO 9637609 and WO 9303162.

Another control means uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated tet
30 R - 4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, *i.e.*, it binds to a tet operator in the presence of tetracycline)

linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Numbers 5,464,758; 5,650,298 and 5,654,168.

Additional preferred methods of expression control are described in Innovir U.S. Patent No. 5,741,679) or the control of gene expression by means of translation inhibition using small molecule RNA interactions; Soukup and Breaker (*Proc. Natl. Acad. Sci. USA* 96:3584-3589, 1999) to construct ligand responsive allosteric ribozymes; WO 0020040 involving the control of gene expression using aptamers; as well copending patent application United States Serial Number 60/186,248 filed March 3, 2000 (Attorney Docket Number A-655-P) which includes descriptions of selection of effectors, *in vitro* evolution techniques, and expression regulation formats using allosteric molecules.

Several *in vitro* evolution (selection) strategies (Orgel, *Proc. R. Soc. London*, B 205: 435, 1979) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as the cleavage and ligation of RNA and DNA (Joyce, *Gene*, 82:83-87, 1989; Beaudry *et al.*, *Science* 257:635-641, 1992; Joyce, *Scientific American*, 267:90-97, 1992; Breaker *et al.*, *TIBTECH* 12:268, 1994; Bartel *et al.*, *Science* 261:1411-1418, 1993; Szostak, *TIBS* 17:89-93, 1993; Kaufmann *et al.* U.S. Patent No. 5,814,476; Kumar *et al.*, *FASEB J.*, 9:1183, 1995; Breaker, *Curr. Op. Biotech.*, 7:442, 1996; and Berzal-Herranz *et al.*, *Genes & Develop.* 6:129, 1992), and new nucleic acids which act as aptamers, such as an ATP aptamer, HIV Rev aptamer, HIV Tat aptamer and others including oligonucleotide effectors (see Ellington and Szostak, *Nature* 346:818-822, 1990; Famulok and Szostak, *Angew. Chem. Int. Ed. Engl.* 31:979-988, 1992; Ellington, *Current Biology*, 4(5):427-429, 1990; Porta and Lizardi, *Bio/Technology* 13:161-164, 1995; Tang and Breaker, *Chemistry & Biology*, 4(6):453-459, 1997; Tang and Breaker, *RNA*, 3:914-925, 1997; Werstuck and Green, *Science*, 282:296-298, 1998; Tang and Breaker, *Nucleic Acids Research* 26(18):4214-4221, 1998; Soukup and Breaker, *Proc. Natl. Acad. Sci. USA*, 96:3584-3589, 1999; and Robertson and Ellington, *Nature Biotechnology* 17:62-66, 1999). In addition to these, further descriptions of *in vitro* evolution processes are provided in Innovir U.S. Patent No. 5,741,679 and U.S. Patent No. 5,834,186, WO 9843993 (PCT/US98/06231, Yale

University), WO 9827104 (PCT/US97/24158, Yale University) and U.S. Patent No. 5,817,785 (NeXstar Pharmaceuticals).

The invention also provides selective binding agents such as monoclonal
5 antibodies, polyclonal antibodies and antibody fragments to PAR-2 receptors. Thus, the present invention further provides a process for the production of monoclonal or polyclonal antibodies to PAR-2 receptors. Preferably, these antibodies are specific for wild type PAR-2 and its isoforms.

The antibodies may be polyclonal including monospecific polyclonal,
10 monoclonal (MAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, and/or bispecific, as well as fragments, variants or derivatives thereof. Antibody fragments include those portions of the antibody which bind to an epitope on the PAR-2 receptor. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments
15 include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

If polyclonal antibodies are desired, a selected mammal (*e.g.*, mouse, rabbit, goat, horse, *etc.*) is immunized by means of multiple subcutaneous or intraperitoneal
20 injections with an immunogenic polypeptide comprising a wild type PAR-2 receptor sequence (epitope(s)) and an adjuvant. It may be useful to conjugate the selected PAR-2 receptor molecule to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance
25 the immune response. Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to a PAR-2 epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the
30 invention also provides polypeptides of the invention or fragments thereof hapttenized to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against PAR-2 receptor molecule or an epitope(s) thereof can also be readily produced by one skilled in the art using any method which provides for the production of antibody molecules by continuous cell lines in culture. The general methodology for making monoclonal antibodies by hybridomas is well known. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, *Nature*, 256:495-497 (1975) and the human B-cell hybridoma method, Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987). Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against orbit epitopes can be screened for various properties; i.e., for isotype and epitope affinity.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. See, U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1985).

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089, and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods described in the art (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting at least a

portion of a rodent complementarity-determining region (CDR) for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies which bind PAR-2 receptor polypeptides. Using transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies are produced by immunization with an PAR-2 receptor antigen (*i.e.*, having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits *et al.*, *Proc. Natl. Acad. Sci.*, 90:2551-2555 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993). In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than *e.g.*, murine) amino acid sequences, including variable regions which are immunospecific for these antigens. See PCT application nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT application nos. PCT/US91/245, PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

An alternative technique involves screening phage display libraries where, for example the phage express scFv fragments on the surface of their coat with a large variety of complementarity determining regions (CDRs). This technique is well known in the art, see, for example, Hoogenboom *et al.*, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991) These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application No. PCT/US98/17364, which describes the

isolation of high affinity and functional agonistic antibodies for MPL- and msk-receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host
5 cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (*e.g.*, human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

Selective binding agents of the invention, including antibodies, may be used as
10 therapeutics. These therapeutic agents are generally PAR-2 antagonists, in that they reduce at least one of the biological activities of a PAR-2 receptor polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to a PAR-2 receptor and which are capable of inhibiting or eliminating the functional activity of a PAR-2 receptor *in vivo*
15 or *in vitro*. In preferred embodiments, the selective binding agent, *e.g.*, an antagonist antibody, will inhibit the functional activity of a PAR-2 receptor by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an antibody that is capable of interacting with a PAR-2 receptor binding partner (a ligand) thereby inhibiting or eliminating PAR-2 receptor activity *in vitro* or
20 *in vivo*. Selective binding agents may be identified by screening assays which are well known in the art.

Antibodies, both monoclonal and polyclonal, which are directed against PAR-2 epitopes are particularly useful in diagnosis. Monoclonal antibodies, in particular, may be used to raise anti-idiotypic antibodies. Anti-idiotypic antibodies are immunoglobulins
25 which carry an "internal image" of the antigen of the agent against which protection is desired. Techniques for raising anti-idiotypic antibodies are known in the art. These anti-idiotypic antibodies may also be useful in therapy.

For the purposes of this invention, the term "antibody", includes fragments of whole antibodies which retain their binding activity for a target antigen. Such fragments

include Fv, F(ab') and F(ab')₂ fragments, as well as single chain antibodies (scFv). Furthermore, the antibodies and fragments thereof may be humanized antibodies, for example as described in EP-A-239400.

- 5 All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference. While this invention has been described with an emphasis upon preferred embodiments, it will be apparent to those of ordinary skill in the art that variations in the preferred embodiments can be prepared and used and that the invention can be practiced
- 10 otherwise than as specifically described herein. The present invention is intended to include such variations and alternative practices. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

CLAIMS

What is claimed is:

- 5 1. A method of treating airway hyperresponsiveness by inhibiting protease activation of proteinase-activated receptor-2 by means of administration of a protease inhibitor.
2. The method of claim 1 wherein the activation of proteinase-activated receptor-2
10 is inhibited by serine leukocyte protease inhibitor (SLPI).
3. The method of claim 1 wherein the activation of proteinase-activated receptor-2 is inhibited by an antibody to PAR-2.
- 15 4. A method of treating airway hyperresponsiveness by inhibiting the activation of proteinase-activated receptor-2 by administering a therapeutic agent to block the proteolytic activation site, the tethered ligand, or the receptor-ligand interaction wherein the therapeutic agent is selected from the group consisting of peptides, antibodies, and small molecules.
- 20 5. The method of claim 4 wherein the activation of proteinase-activated receptor-2 is inhibited by a small molecule to block the proteolytic activation site, the tethered ligand, or the receptor-ligand interaction.
- 25 6. A method of treating asthma comprising delivering to the patient an inhibitor or antagonist of proteinase-activated receptor-2 and thereby decreasing airway hyperresponsiveness.
7. A method for identifying molecules useful in the treatment of airway
30 hyperresponsiveness, comprising coincubating a selected molecule and a cell expressing a proteinase-activated receptor-2, wherein the molecule is being evaluated as

a potential proteinase-activated receptor-2 antagonist or inhibitor and determining whether proteinase-activated receptor-2 autoactivation is inhibited or antagonized.

8. A method according to claim 7, wherein said cell is selected from the group
5 consisting of endothelial cells, keratinocytes, aorta cells, coronary artery cells, intestinal artery cells, smooth muscle cells, epithelium cells, sweat gland cells, vascular smooth muscle cells, astrocytes and T-lymphocytes.

9. A method for identifying a proteinase-activated receptor-2 antagonist or
10 inhibitor, comprising coincubating a molecule and a cell expressing proteinase-activated receptor-2 and determining whether proteolytic cleavage and activation of proteinase-activated receptor-2 is inhibited or antagonized.

10. A method according to claim 9, wherein said cell is selected from the group
15 consisting of endothelial cells, keratinocytes, aorta cells, coronary artery cells, intestinal artery cells, smooth muscle cells, epithelium cells, sweat gland cells, vascular smooth muscle cells, astrocytes and T-lymphocytes.

11. A method of identifying a molecule that modulates the activity of proteinase-
20 activated receptor-2 which method comprises coincubating smooth muscle tissue with a candidate molecule and determining whether the candidate molecule modulates the activity of said proteinase-activated receptor-2 by assessing a change in smooth muscle responsiveness to a proteinase-activated receptor-2 agonist and selecting those
25 candidate molecules that significantly inhibit the activation of proteinase-activated receptor-2.

12. The method of claim 11 wherein said smooth muscle tissue is bronchial tissue.

13. The method of claim 11 wherein said proteinase-activated receptor-2 agonist is
30 selected from histamine, serotonin and methacholine.

14. The method of claim 11 wherein the assessment of smooth muscle responsiveness comprises the measurement of the isometric contractions of said smooth muscle tissue.
- 5 15. The method of claim 11 wherein said molecule is selected from the group consisting of peptides, antibodies and small molecules.
16. A compound identified by the method of claim 11, 12, 13, 14 or 15.
- 10 17. A method of identifying a molecule that modulates the activity of proteinase-activated receptor-2 which method comprises coincubating endothelial cells with a candidate molecule and determining whether the candidate molecule modulates the activity of said proteinase-activated receptor-2 by assessing a change in interleukin-8 production and selecting those candidate molecules that inhibit interleukin-8 production
- 15 thereby indicating anti-inflammatory activity.

FIGURE 1

PAR-2 Specific Induction of Guinea Pig Bronchi Hyperresponsiveness to Histamine

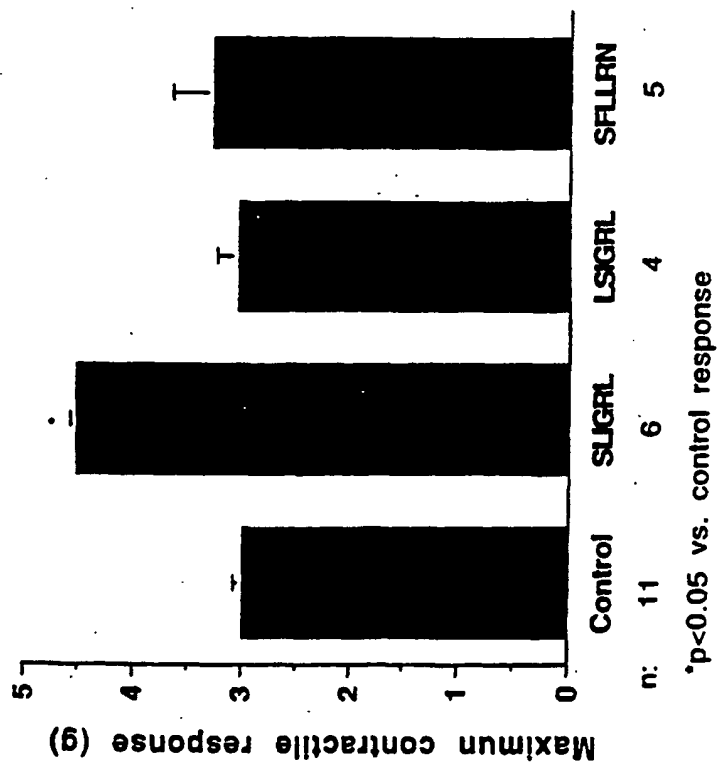
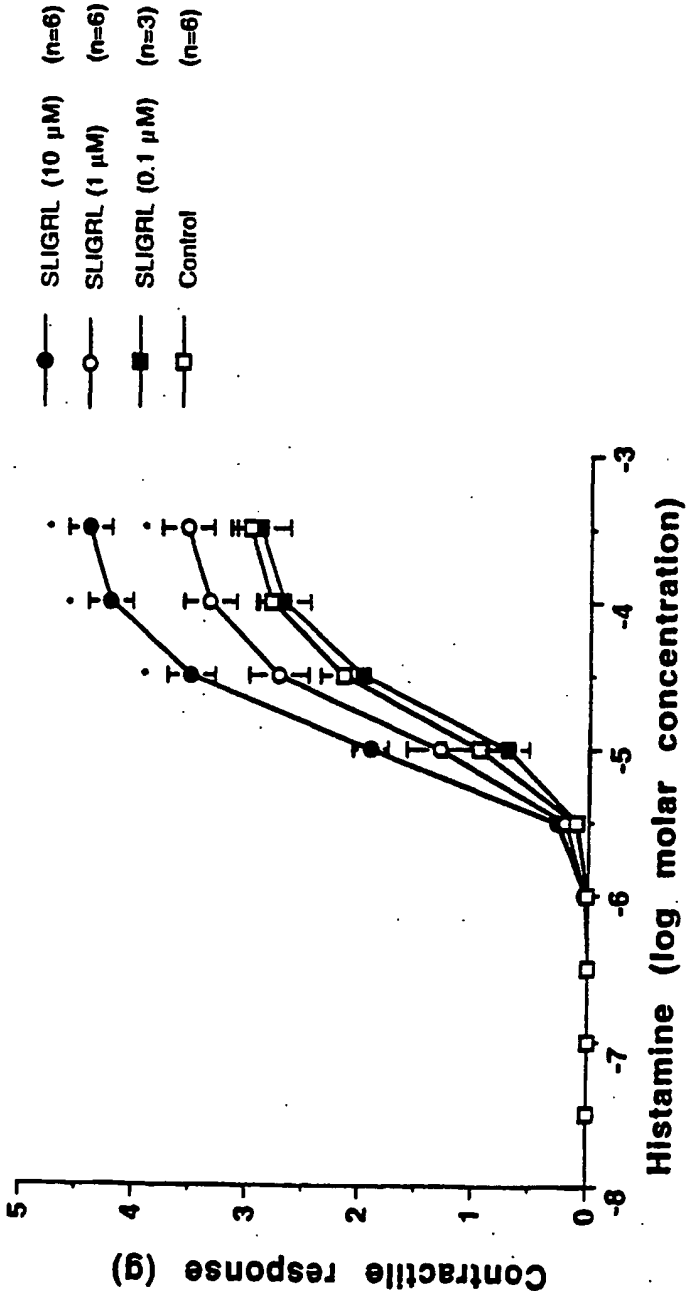


FIGURE 2a
Effect of PAR-2 Agonist on Contractile Response
of Guinea Pig Bronchi to Histamine



*p<0.05 vs.. control response

FIGURE 2b
Effect of PAR-2 Agonist on Contractile Response
of Guinea Pig Bronchi to Histamine

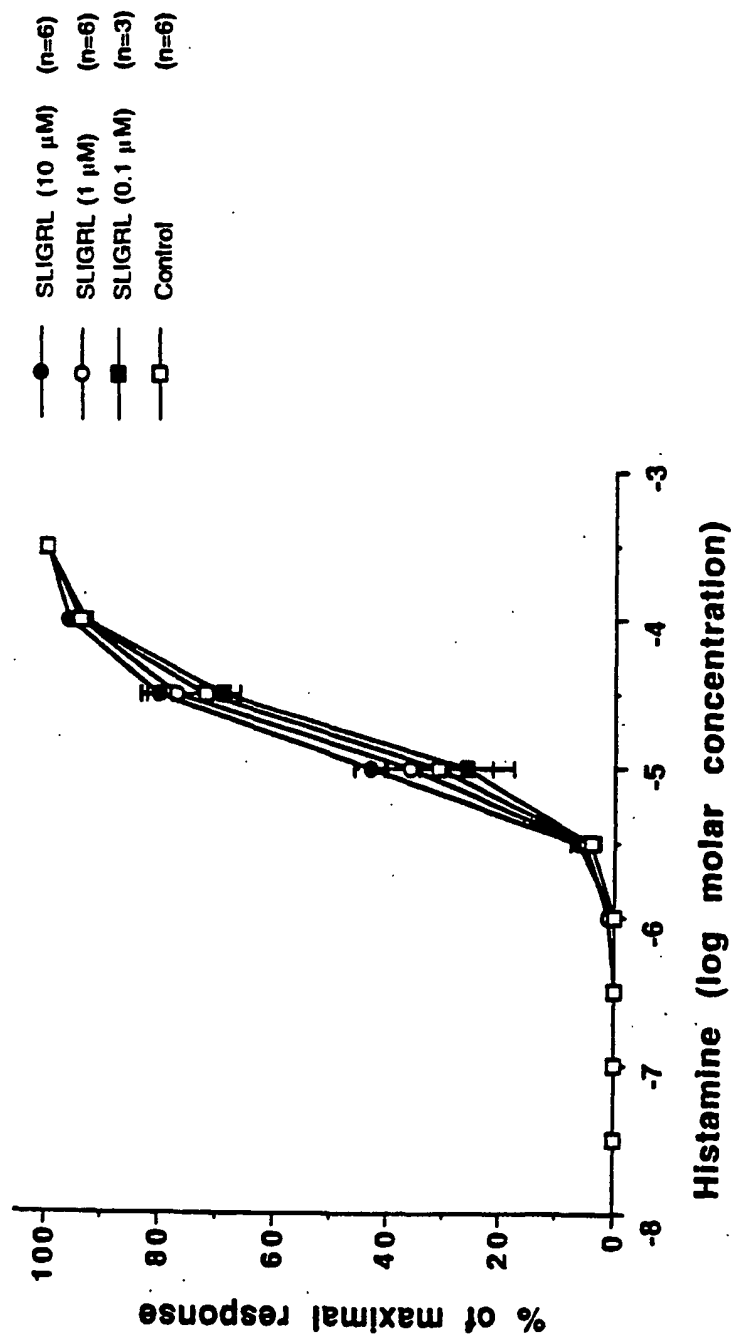


FIGURE 3

Time-Dependent Induction of Guinea Pig Bronchi Hyperresponsiveness by the PAR-2 Agonist SLIGRL

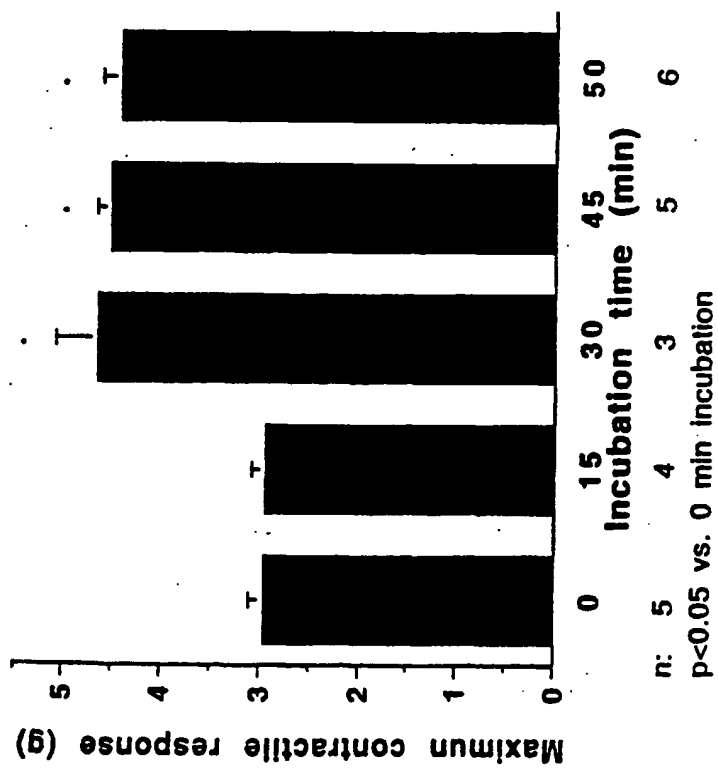


FIGURE 4
SLPI and APC366 Do Not Inhibit SLIGRL-Induced
Airway Hyperresponsiveness

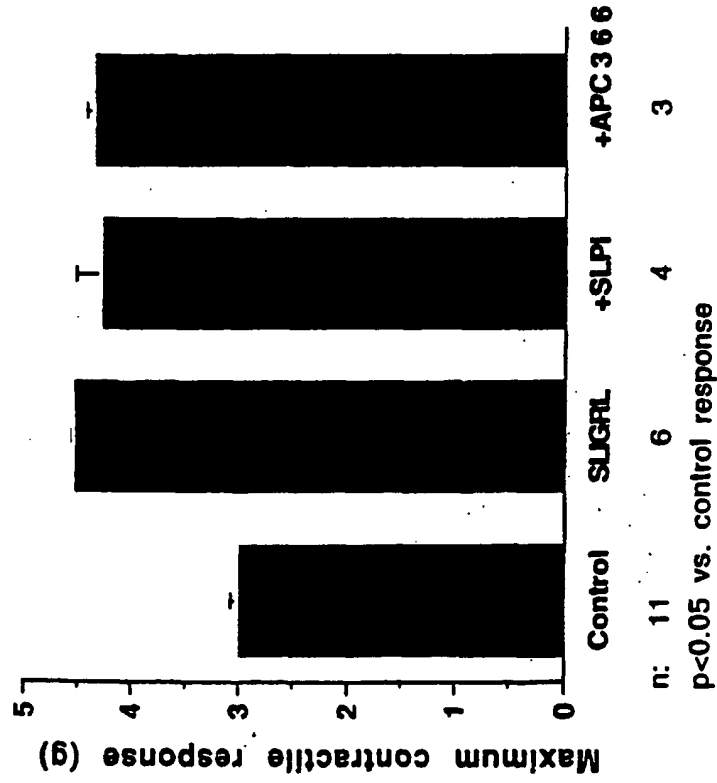


FIGURE 5

Effect of Indomethacin on Induction of Guinea Pig Bronchi Hyperresponsiveness by the PAR-2 Agonist SLIGRL

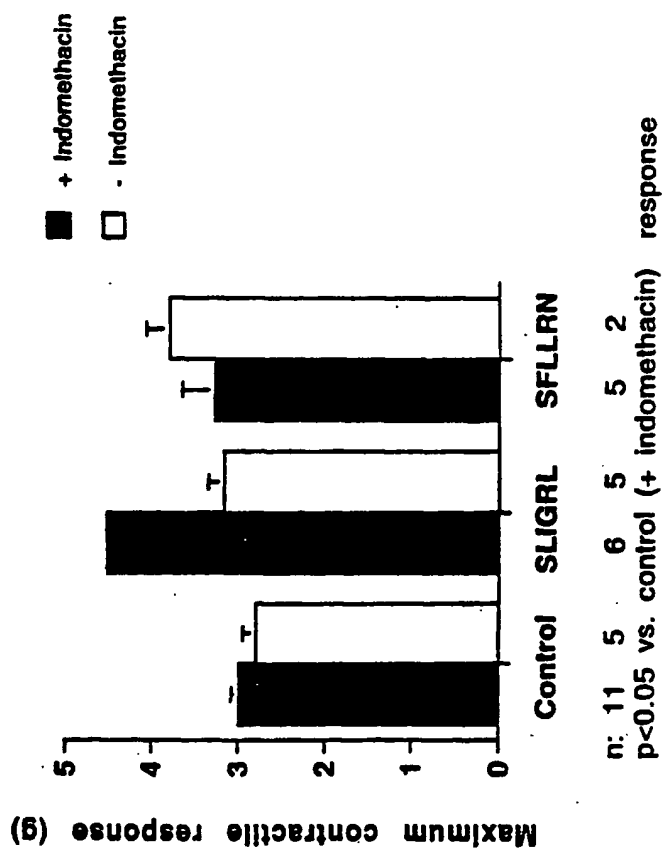
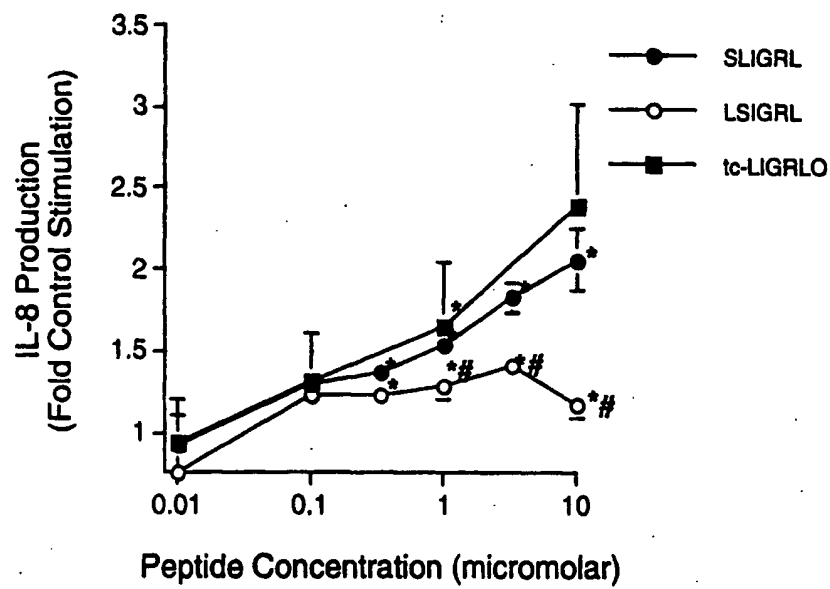


Figure 6**PAR-2 Agonists Stimulate IL-8 Production by
Human Umbilical Vein Endothelial Cells**

*p<0.05 versus unstimulated cells

#p<0.05 versus SLIGRL-stimulated cells

SEQUENCE LISTING

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|

<120> INHIBITORS OF PROTEASE-ACTIVATED RECEPTOR-2 (PAR-2) AS
NOVEL ASTHMA THERAPEUTICS

<130> A-656A

<140> Not Yet Assigned

<141> 2000-11-28

<150> 60/177,137

<151> 2000-01-20

<150> 60/203,357

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/01968

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, PHARMAPROJECTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 09297 A (ARRIS PHARM CORP) 28 March 1996 (1996-03-28) *cf. abstract, p. 3, last para. bridging with page 4, first para., page 9, last para. and page 10, lines 1-2*	1-17
Y	WO 99 17800 A (AMGEN INC) 15 April 1999 (1999-04-15) *cf. abstract, page 3, last para. with page 4, lines 1-3, page 8, lines 11-22*	1-17
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Z document member of the same patent family

Date of the actual completion of the international search

25 May 2001

Date of mailing of the international search report

07/06/2001

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/01968

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	JP 06 228000 A (TEIJIN LTD) 16 August 1994 (1994-08-16) *cf. abstract* ---	1-17
Y	WO 98 43477 A (UNIV CALIFORNIA) 8 October 1998 (1998-10-08) *cf. abstract, page 2, lines 18-35, page 4, lines 20 bridging with page 5, lines 1-21, page 9, last para. and page 10, lines 4-15* ---	1-17
Y	WO 96 08275 A (BAYER AG) 21 March 1996 (1996-03-21) *cf. abstract, page 3, lines 3-15* -----	1-17

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Information on patent family members

International Application No

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